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# Larvicidal, ovicidal and repellency properties of *Citrus aurantifolia* (Lemon) peels essential oil against *Aedes aegypti* mosquitoes

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## ABSTRACT

Mosquito-borne diseases are mostly harmful to children being and it is a public health problem in Myanmar. Laboratory reared Dagon Myothit North strain of *Aedes aegypti* larvae were used to test larvicidal, ovicidal and repellency properties of *Citrus aurantifolia* (Lemon) peels essential oil from June 2021 to May 2022 according to WHO. Fresh peels 300 grams from Taikkyi was extracted by stream distillation at 100°C for 3hours and obtained 1.65g of essential oil. Different concentrations of Lemon peels essential oil were prepared freshly in 100ml each of distilled water in 150ml plastic cups. Fifty each *Aedes* larvae were exposed 24hrs for each replication in different concentrations in laboratory. Acute toxicity and allergenicity tests were done in laboratory according to OECD Guidelines. Repellency test was done by laboratory reared 5-7 days old adult female *Aedes* mosquitoes with Lemon peels essential oil. Results revealed that the highest dose 0.01g of Lemon peels essential oil produced 100% knockdown within 60minute and 98.8% mortality within 24hrs respectively and 100% ovicidal effect for 4days as well as persistency was observed 98.8 - 100% mortality of larvae for 4 days. The effective lethal concentrations $LC_{50}$ and $LC_{90}$ values were found to be 0.0017g and 0.0053g of peels essential oil (x=0.05236, P=0.05). There was not found any acute toxicity on mice and allergenicity on the rabbits. 100% protection of *Aedes* mosquito landing to probe the skin was found 0.04g/ml or 0.000128g/$cm^2$ of essential oil. Repellency activity of complete protection time was observed over 80% protection for 210minutes, over 90% prevention for 150 minutes, and 100% prevention for 120mins. Semi field trial observed that in the day time, it can prevent 3 hours of *Aedes* mosquito bite on oil applied areas of insect collectors in household. The essential oil is not toxicity, no allergenicity and no irritation of skins of animal. Therefore, the cheap, effective, ecofriendly and degradable lemon *Citrus aurantifolia* peels essential oil can be used as insecticide and repellent of mosquitoes in public sector.

**Keywords:** Allergenicity; *Aedes aegypti*; *Citrus aurantifolia*; Dilution; Essential Oil; Knockdown; Lemon Peel; Larvae; Larvicidal; Mosquitoes; Mortality; Ovicidal; Persistency; Protection Time; Repellency; Toxicity Test.

## 1.0. Introduction

Dengue Fiver (*DF*) and Dengue Hemorrhagic Fever (*DHF*) are mail problem of mosquito borne diseases in Myanmar. The mosquito *Aedes aegypti* and *Aedes albopictus* are considerable public health importance worldwide, mainly due to its involvement in the transmission of RNA virus belonging to the family Flaviviridae and genus Flavivirus arboviruses (*Orthoflavivirus dengue*), it exists as four distinct, related serotypes DENV-1, DENV-2, DENV-3, DENV-4 viruses).

Mostly *Aedes* mosquitoes (*Aedes aegypti* and *Aedes albopictus*) transmits Dengue Fiver (*DF*) and Dengue Hemorrhagic Fever (*DHF*), Dengue Shock Syndrome (DSS) Yellow fever, Zika, Chikungunya, *Culex mosquitoes* (*Culex quinquefastiatus* transmits Filariasis, and *Cx. tritaeniorhynchus* transmits Japanese encephalitis, and *Anopheles* mosquitoes (*Anopheles dirus, An. minimus* are main vectors, and *An. annularis, An. sundiagus* are local vectors) transmits malaria in Myanmar and other *Anopheles* species such as *An. philippinensis, An. culicifacies, An. stephensi* are vector of malaria in India [1-5].

*Aedes aegypti* is a Urben vector and *Ae. albopictus* is a Rural vector and Other *Aedes* species as *Ae. cogalli* was first collected in Yee Township Mon State and the species now spread to Kayin State but not a vector of dengue, although insecticide resistant was found in an African origin India and Sri Lanka [6-8].

## 2.0. Literature

*Ae. aegypti* is a African origin and present still now, reproducing in forests and ecotones, where adult's mosquito prefer wild animals for blood meal and larvae were bred in tree holes [9,10]. African villages and cities were main mosquito breeding sites during dry seasons because people there started keeping water in containers when they first settled there [11].

In Myanmar *Ae. aegypti* and *Ae. albopictus* vectors of Dengue and Dengue Heamorrigic Fever and first-time report of severe dengue out brake was observed in 1970 in Yangon and now, it was gradually spread whole of the country [12]. The highest number of cases and deaths were recorded 9149 *DHF* cases and 55 deaths were recorded across Myanmar [13]. Dengue Fever (*DF*), Dengue Hemorrhagic Fever (*DHF*) and Dengue shock syndrome (*DSS*) are increasingly becoming serious public health problem in Myanmar, [14]. A vast majority of the *DF* and *DHF* cases occur in 5-8 years and harmful among the 5-15 years old age groups and now noted 15years above [15,16]. In year 2006, Indonesia reported quite a number of dengue cases in South East Asian region, with 57% contributing to the southern hemisphere countries [17].

Indonesian government has focused on vector control management by biological, chemical or environmental management using larvicides as a complementary way in eradicating dengue vector development according to the World Health Organization (WHO) guideline. One percent has been used for resistance management tool in mosquito abatement programs. However, it also causes toxicity in higher dosage due to overstimulation of the nervous system in humans [18,19]. Thus, alternative natural phytochemicals are preferred to encounter toxicity problems.

Secondary metabolites were playing an important role in plant defense system as well as in its relationship with other plants, herbivores, pathogens and pollinators [20]. Essential oil (EO)s are aromatic, liquefied, and volatile substances which are produced by different plant parts as leaves, flowers, seeds, buds, twigs, barks, herbs, woods, fruits and roots and they are the complex natural mixtures of lipophilic substances [21], containing about 20–60 compounds, two or three major compounds accounting 20–70% of the total oil compared to others. At present, approximately 300 Essential Oils are commercially available for pharmaceutical, food, sanitary, cosmetic including perfume industries [20], Essential Oil has been getting amazingly useful in the industries like flavoring and fragrance along with pharmaceutics due to their high aroma. Not only in the perfume industry, many study on effectiveness of Essential Oils have been done their antibacterial, antiparasitic, antifungal and insecticidal activities in laboratory and fields [22-24].

Lemon *Citrus aurantifolia* (Lemon) is a plant native to tropical Asia, Africa and the Pacific Islands. Fruit peels contain a high percentage of volatile and essential oils and is used in the conventional medicine to alleviate pain, vomiting and even as an insect repellent [25]. *Citrus aurantifolia* is a genus Citrus consisting of chemical coumarins such as phenolics, scopoletin, flavonoids and limonin, are very effective phytochemical larvicides [26, 27]. Research in Thailand and Myanmar has shown that *Citrus hystrix's* fruit peel has higher mortality compared to the plant leaves against *Aedes aegypti* larvae [8]. A study in Hpa-a Township, Kayin State, revealed that the biological control of *Aedes* larvae in water storage containers using neem (*Azadirachta indica*) seed oil was very effective to control *Aedes* larvae as larvivorus fish [28,29].

Synthetic insecticides are toxic and adversely affect the environment by contaminating soil, water, and air and human being [30,31]. There is a need to find out the alternative ways for environmental safety, biodegradable, low cost and indigenous methods for vector control.

Larval and pupal life stages of mosquitoes are mostly confined to tropical and temperate waterbodies and often form a significant proportion of biomass waterbodies. Due to rebound vectorial capacity, resistance to chemical insecticides, and harmful environmental effects, the vector control program has shifted to using biological control agents. These methods are target-specific, eco-friendly, cost-effective, and can be easily deployed. Therefore, the study was assessed the larvicidal, ovicidal and repellency properties of the indigenous fruit *Citrus aurantifolia* (Lemon) peels essential oil against *Aedes aegypti* mosquitoes.

## 3.0. Aim of the study

To assess the larvicidal, ovicidal and repellency properties of the indigenous fruit *Citrus aurantifolia* (Lemon) peels essential oil against *Aedes aegypti* mosquitoes.

### 3.1. Specific Objectives

1. To explore the larvicidal, ovicidal and repellency effect of indigenous botanical plants (*Citrus aurantifolia*) fruit peels essential oil for the control of DHF vector *Aedes aegypti*.
2. To determine the corrected lethal concentration for controlling *Aedes* larvae.
3. To discover the probable use of the essential oil as a potential repellent agent against *Ae. aegypti* mosquitoes.

## 4.0. Materials and Methods

**4.1. Study Design**: Laboratory and semi field base experimental study.

**4.2. Study Period**: Study was started from June 2024 to complete May 2025.

**4.3. Study area**: The study was conducted in Medical Entomology Research, Department of Medical Research No. (5) Ziwaka Road Yangon Taikkyi and Dagonmyothit North Townships, Yangon Region to do Larvicidal, Ovicidal and Repellent properties of *Citrus aurantifolia* peels essential oil.

**4.4. Study population:** At least 5000 larvae, 1000 eggs and 3000 adults of *Aedes aegypti* mosquitoes were used to do the experiment in laboratory.

**4.5. Sample size and sampling**: Three kg of Lemon peels was collected from Taikkyi Township, Yangon Region. Dried it under shaded condition in laboratory. Dried materials were kept air tight tin can in Air conditional room till to extract the essential oil.

*Aedes aegypti* larvae were collected from water storage containers in Dagonmyothit North Township Yangon Region. All collected larvae and pupae were put into plastic bags and supplied Oxygen by Oxygen pump and carried by Car to Medical Entomology Research Division, Department of Medical Research for colonization and further study.

**4.6. Species Identification**: Species identification of collected larvae and adult were done according to Rumpa and Prachong [32].

**4.7. Ethical consideration**: DMR Ethical Review Committee. Approval Certificate Number….2022/25.


Study sites Union of Myanmar

**4.8. *Citrus* peels essential oil extraction:** Clean dried 300grams of *Citrus aurantifolia* peels +2.5liters distilled water was extracted by stream distillation at 100°C for 3 hours and essential oil was obtained after evaporation of water by rotary evaporator and 8gm of essential oil was obtained.

**4.9. Toxicity and Irritation test of *Citrus aurantifolia* fruit peels essential oil**

**4.9.1. Toxicity test**

**4.9.1.1. Acute toxicity test of the samples on albino mice model**

**Theory**

To determine the symptomatology consequent to injection of the plant and to determine the nature and degree of toxicity produced by these extracts and to find out the medium lethal doses ($LD_{50}$) of the extracts, acute toxicity test was done. Usually, the acute lethality a compound is determined on the basic of deaths occurring in 24 h but the survivors should be observed for at least seven days in order to detect delayed effects. In this study, acute toxicity effect of *Citrus aurantifolia* fruit peels essential oil was determined on albino mice, at Laboratory Animal Services Division, Department of Medical Research (DMR), Yangon.

**4.9.1.1.1. Procedure**

Acute toxicity of different doses *Citrus aurantifolia* fruit peels essential oil of that samples were evaluated by the methods of OECD Guidelines for the testing of Chemicals 425 [33]. According to the test description, total number of 18 adult female albino mice, weighting (25-30g) was selected and divided into three groups. Each group contained six animals. They were fasted for 18 h before giving the essential oil. Group (1) mice were orally administrated with 2000 mg/kg dose of *Citrus aurantifolia* fruit essential oil. Group (2) mice were given orally with *Citrus aurantifolia* fruit peels essential oil 5000 mg/kg dose. Group (3) mice performed as a control group and they were treated with clean water and normal animal food. All groups of mice were kept in the three mouse cages in the

separated room at the room temperature of 26 ± 1º C. After administration of extracts on each group of animals were observed first 6 h continuously for mortality and behavior changes. Then check the animals each 24 h for fourteen days. The mortality during this period was noted (Nil or percent death). The results obtained from acute toxicity were recorded. Same toxicity test procedure for *Citrus aurantifolia* fruit peels essential oil was followed as above mention procedure.

**4.9.1.1.2. Experiment of irritation test**

Primary skin irritation is the production of reversible inflammatory changes in the skin following the application of a test substance as it involves the interaction of chemicals with the sensory receptors in the skin at the site of application. Skin irritation test was done according to 'DBT, Guidelines for toxicity and allergenicity [33]. In the present study three young adult rabbits of the Myanmar white strain were taken from animal service division, two samples for test and one sample for control. All animals were housed in metal cages fitted with perforated floors. Water and standard rabbits feed were given. The room temperature was maintained at 22 ± 3 ° C with 30 - 70 % relative humidity. The light conditions were controlled to give 12 hours artificial light (8 a.m. - 8 p.m.) each day. A minimum of 7 days acclimatization was allowed before the commencement of the study. Each rabbit cage was attached with a tag marked with the animal number, the test and the product name.

Twenty-four hours before the test (dose application), hair on the back of each rabbit was shaped by blade approximately 9 $cm^2$ area of skin. (DOSE: 0.0036 $gm/cm^2$ area of rabbit = double dose of human 0.0002$g/cm^2$), 0.0072gm of *Citrus aurantifolia* fruit peels essential oil was desorbed in 2ml of ethanol and this 1ml each mixture dose form was evenly applied to a small area (approximately 9 cm square) of the shaped skin of each test rabbit. The site of application was not covered with a cotton gauze patch. Similarly control rabbit was treated with only 1ml of ethanol. Skin reaction at the site of application was subjectively assessed and scored once daily at 1, 24, 48, 72 hours, 7 and 14 days after treatment of skin (post-test observation period) according. The reaction at the site of application was assessed and scored according to the following numerical system. : Skin reaction: (A) Erythema and Escher formation (B) Edema formation. Irritation was followed by evaluation of primary skin irritation index: Non-Irritant 0.0, Negligible Irritant 0.1- 0.4, Slight Irritant 0.41-1.9, Moderate Irritant 2.0 - 4.9, Severe Irritant 5.0 - 8.0. Same above procedure of irritation test was followed for irritation testing of *Citrus aurantifolia* fruit peels essential oil on other rabbits.

**4.9.1.2. Larvicidal and repellency tests:** After preliminary larvicidal test of *Aedes* larvae, 50 3rd and 4th instar *Aedes aegypti* larvae each were exposed in *Citrus aurantifolia* peels essential oil 0.01,0.005, 0.0025,0.00125,0.000625g concentrated in 100ml distilled water for 24 hours. Knockdown effect of larvae was measured after 60 minutes of exposure period and mortality was calculated after 24hours exposure period [34].

**4.9.1.3. Larvicidal and Ovicidal persistency test**: Larvicidal and Ovicidal persistency test was done in Laboratory, daily 20 3rd and 4th instar *Aedes* larvae and 20 fresh *Aedes* eggs each were put into 0.01g/100ml concentrated water individually for 6days and mortality was calculated after 24 hours daily [34].

**4.9.1.4**. **Repellent activity testing**

The repellent study was followed the method of WHO [35]. Five days old blood staved female *Aedes aegypti* mosquitoes 60 each were kept in two steel net cages (59x59x59cm) one for male and one for female volunteers.

*Aedes aegypti* is a day time biter; therefore, the tests were done between 08:00 hour and 16:00 hour. Evaluation tests were carried out in a (12x15x15ft) room at 24-28°C and relative humidity of 70-85%. The volunteers had no contact with lotions, perfumes, or perfumed soaps on the day of assay. The arms of volunteers, one ml of ethyl alcohol 95% diluent used in the preparation of the test repellent in applied evenly using a pipette to average 372.375cm2 (Four volunteers-two males +two female) of forearm skin between the wrist and elbow and allowed to dry for 1 minute. Before insertion of arm into the cage containing 60 *Aedes* female mosquitoes, the hands are protected by plastic gloves to protect mosquito bite. The first step, ethyl alcohol applied forearm was inserted into the cage and counted the number of mosquitoes that land on skin during 30-second period. The control forearm was carefully withdrawn and this arm was then treated with one ml of 0.04g/ml or 0.000128g/cm$^2$ of essential oil solution and allowed to dry. The treated arm was placed in the cage for another 30 second period and on served for mosquito landing. This procedure was repeated for each additional incremental of *Citrus aurantifolia* peel essential oil dose. The tests were carried out one after the other without delay and *Citrus aurantifolia* fruit peels essential oil dose at each test was calculated as the sum of the doses applied to arrive at the cumulative dose for each test. Test was proceeding when the mosquito landing rate on the exposed forearm was less than 10 females in 30 second. Two trained technicians were recorded the number of landings. At the conclusion of the dose response experiment, 1ml of ethyl alcohol was applied on the other forearm and allowed to dry. This forearm was inserted in the cage for 30 second to verify that the number of landings was more than 10 per 30 second as was observed at the beginning of the experiment. Protection (P) was expressed as a proportion of the number of mosquito landing on treated arm (T) in relation to the number of landings on the control arm C of the same individual. Where C is the average of landing mosquitoes on two un treated arms.

P+1-(T/C)=(C-T)/C

**4.9.1 5. Estimation of complete protection time**

The complete protection time of *Citrus aurantifolia* fruit peels essential oil was determined 99 to 100% protection dose 0.04 g/ml of essential oil was using on average 372.375cm$^2$ areas (another 2 male and 2 female volunteers) of forearm skin between the wrist and elbow individually. The period of protection test was followed by the procedure described as above. Although, Oil applied hand and only Ethanol applied hands were exposed for 3minutes in the mosquito cage.

Before testing, two mosquito cages (size 59x59x59cm) each containing 60 non-blood fed 5 days old *Aedes aegypti* female mosquitoes were normally used. One cage was used for testing female volunteers and another cage was used for testing male volunteers. Before testing the arms of volunteers, one ml of ethyl alcohol 95% diluent used in the preparation of test of *Citrus aurantifolia* peels essential oil repellent in applied evenly using pipette to average 372.375cm$^2$ of forearm skin between the wrist and elbow and allowed to dry 1 minute. Before insertion of arm into the cage containing 60 *Aedes* female mosquitoes, the hands are protected by plastic gloves to protect mosquito bite.

**4.9.1.5.1. Procedure**

The first step, 1 ml ethyl alcohol only applied forearm was inserted in to the cage and counted the number of mosquitoes that land on the skin during 3minute period and counted for mosquito landing. The control forearm was carefully withdrawn from the cage.

Then 0.04g of *Citrus aurantifolia* fruit peels essential oil was prepared in one ml of ethyl alcohol solution was applied evenly on average 372.375cm$^2$ of another forearm skin between the wrist and elbow and the hands are protected by plastic gloves to protect mosquito bite. The treated arm was placed in the cage for 3minute period and counted for mosquito landing.

After 30minutes, the *Citrus aurantifolia* fruit peels essential oil repellent treated arm was inserted again into the cage and exposed for 3 minutes to determine landing activity. This procedure was repeated at 30minute intervals for 240minutes and the procedure was used consistently throughout the experiment. Test was repeated 4 times for 2 males and 2 females' volunteers. The mosquitoes that landed on the hand were recorded and then shaken off before imbibing any blood. Test was proceeding when the mosquito landing rate on the exposed forearm was less than 80% within 3 minutes. The end of the experiment, ethyl alcohol applied forearm was inserted into the cage again and counted the number of mosquitoes that land on the skin during 3-minute period to verify that the number of landings was same or not observed at the beginning of the experiment. The control forearm was carefully withdrawn from the cage. The average of landing mosquitoes on two untreated arms was used to calculate the percentage protection of mosquito bite. Complete protection time was estimated after experiment.

### 4.10. Data analysis plan

Data entry and proceeding was made using Microsoft Excel software. The average larval mortality data were subjected to probit analysis for calculating $LC_{50}$ and $LC_{90}$ values and other statistics at 95% confidence limits and Chi-squire values were calculated using the dose –effect probit analysis [36]. Results with $p<0.05$ were considered to be statistically significant.

## 5.0. Results

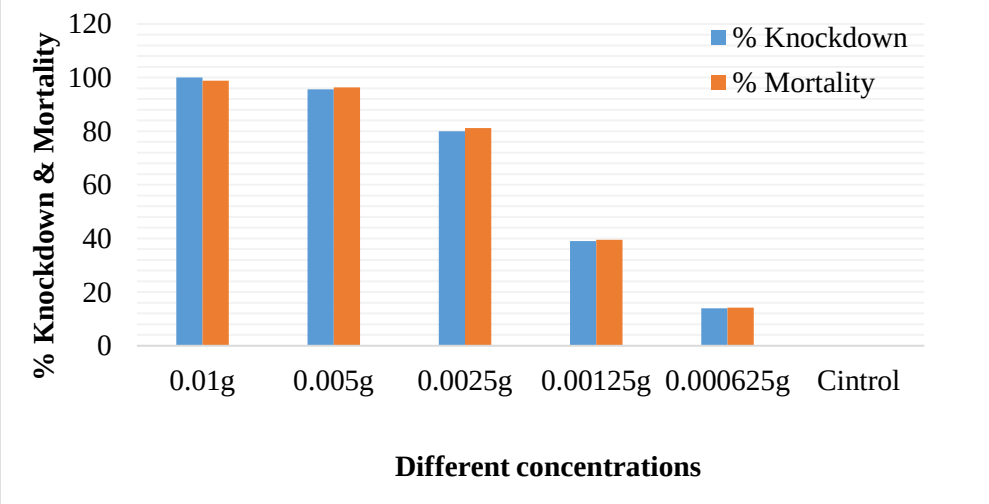


**Figure 1.** Knockdown and mortality effect of different concentrations of *Citrus aurantifolia* peels essential oil against 3$^{rd}$ and 4$^{th}$ instars *Aedes* larvae

Figure 1. shows that *Aedes aegypti* larvae (3$^{rd}$ & 4$^{th}$ instar) from Dagonmyothit North were very susceptible to 0.01g of *Citrus aurantifolia* peels essential oil, 100% knockdown within 60 minutes and 98.8% mortality within 24hrs. followed by 0.005g dose was found 95.6% Knockdown and 96.4% mortality and lowest was 0.000625g dose observed 14%Knockdown and 14.2% mortality.

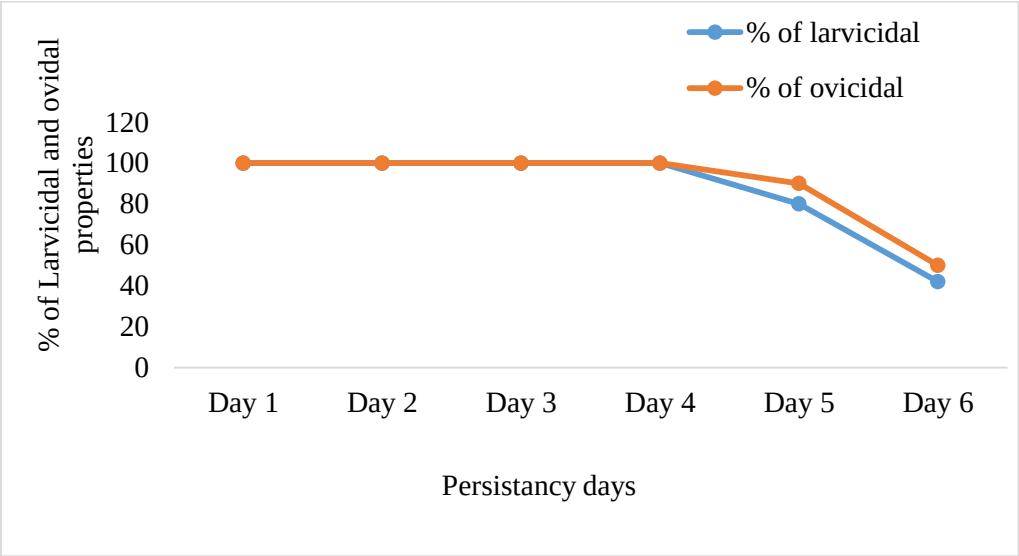


**Figure 2.** Larvicidal and ovicidal persistency of 0.01g *Citrus aurantifolia* peels essential oil against 3rd and 4th instar *Aedes* larvae and eggs

Figure 2. Shows that 0.01 g of *Citrus aurantifolia* peels essential oil against 3rd and 4th instar *Aedes* larvae and eggs found 100% ovicidal and larvicidal persistency effect for 4 days in laboratory condition. At the fifth day, 80% of eggs were destroyed and 20% of the eggs were hatched to 1st instar larvae and 3rd and 4th instar larvae were found 90% mortality and 10% was found alive. Persistency of 0.01g in 100 ml water can persist for 4 days

**Table 1.** $LC_{50}$ and $LC_{90}$ values of *Citrus aurantifolia* peels essential oil on 3rd and 4th instar *Aedes aegypti* larvae

| **Treatment** | **Hours** | **Oil** | **$X^2$, P<0.05** | ***Df*** | **Corrected Lethal Concentration $LC_{50}$** | **Corrected Lethal Concentration $LC_{90}$** |
|---|---|---|---|---|---|---|
| *C. aurantifolia* peels essential Oil | 24 | Watery extract | $X^2$=0.5236 P<0.05 | 4 | 0.0017g | 0.0053g |

$X^2$=Chi squire, P=probability, df= degree of freedom.

Table 1 shows that dose effect analysis of $LC_{50}$ and $LC_{90}$ values of *Citrus aurantifolia* peels essential oil against 3rd and 4th instars *Aedes aegypti* larvae were found 0.0017g for 50% mortality and 0.0053g for 90% mortality, respectively, p<0.05, X2=0.05236.

**Table 2.** Acute toxicity effect of *Citrus aurantifolia* peels oil on albino mice model after two weeks of administration

| **No.** | **Groups** | **Essential Oil Administration** | **Dosage** | **No. of Death** | **% of Death** |
|---|---|---|---|---|---|
| 1 (10 mice) | Group 1 | *Citrus aurantifolia* Peels Essential Oil | 2000 mg/Kg | Nil | 0% |
| 2 (10 mice) | Group 2 | *Citrus aurantifolia* Peels Essential Oil | 5000 mg/Kg | Nil | 0% |
| 3 (10 mice) | Group 3 | No Administration | Nil (Clean water) | Nil | 0% |

Table 2. shows that Acute toxicity effect of *Citrus aurantifolia* peels oil dose 2000mg/Kg and high dose 5000mg/Kg on albino mice model after two weeks of administration were found no anyone of mice was dead, that mean no Acute toxicity effect on albino mice model.

**Table 3.** Allergenicity effect of Lemon peels essential oil reactions on rabbits' skin

| Hours | Combined Index | Edema score | Average score for test | Erythema score | % Score & Index |
|---|---|---|---|---|---|
| 1 hr. | 0 | 0 | 0 | 0 | 0 |
| 24 hr. | 0 | 0 | 0 | 0 | 0 |
| 48 hr. | 0 | 0 | 0 | 0 | 0 |
| 72 hr. | 0 | 0 | 0 | 0 | 0 |
| 7days | 0 | 0 | 0 | 0 | 0 |
| 14days | 0 | 0 | 0 | 0 | 0 |

Table 3 shows that Allergenicity effect of Lemon peels essential oil reactions on rabbits' skin and found that there was not found allergenicity effect of *Citrus aurantifolia* Lemon peels essential oil reactions on rabbits after applied of Lemon peels essential oil on Rabbits skin between 1 hour to 14 days trial.

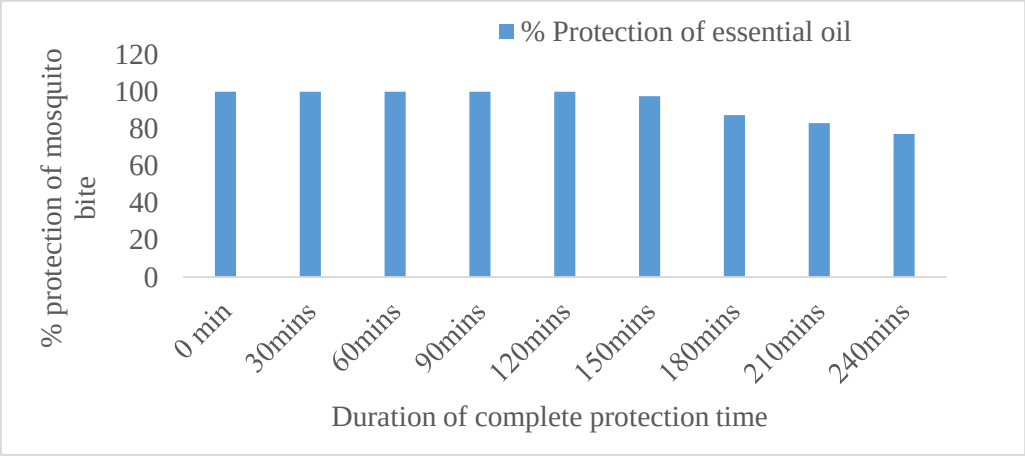


**Figure 3.** Complete protection time of Lemon peels essential oil against *Aedes* mosquito bite.

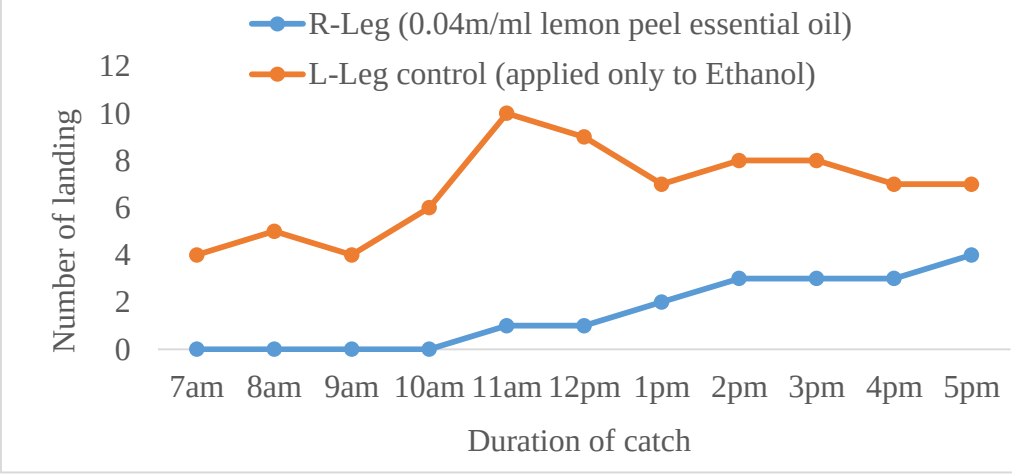


**Figure 4.** Hourly mean landing rate of mosquitoes against Lemon peels essential oil and only Ethanol applied areas of insect collators in semi field area.

Figure 3 shows that 0.04g /ml or 0.000128g/cm2 of *Citrus aurantifolia* peels essential oil was found very effective to repel and protect 80 to 100% protection of *Ae. aegypti* mosquito bite for 210 minutes and 100% protection of bite for 120mins and over 90% protection of mosquito bite was observed for 180mins.

Figure 4. Semi field trial observed that in the daytime, 100% prevention for 3 hours of *Aedes* mosquito landing on oil applied areas of insect collectors in household. After three-hour, landing of mosquito was gradually increased from 1to 4 *Aedes* mosquito landing on oil applied right-Leg, although oil not apply left leg was found high number of mosquito lading.

## 6.0. Discussion

Dengue fever (DF), Dengue Hemorrhage fever (DHF) and Dengue shock syndrome (DSS) are caused by 4 types of dengue virus diseases, transmitted by two main vectors of *Aedes aegypti* and *Aedes albopictus* in Myanmar. The highest number of cases recorded was 43,485 in 2015 and highest number of deaths recorded was 444 in 1994 [37]. *Ae. aegypti* is a main vector in urban areas *Ae. albopictus* is a secondary vector and highly found in rural areas in Myanmar [6,28]. Natural phytochemical larvicide is becoming a complementary way for vector control management. The *Citrus* fruit essential oil has natural chemical reactions against mosquito larvae and adults. This study aimed to identify the larvicidal, ovicidal and repellency activity of *Citrus aurantifolia* fruit peels essential oil against larvae, eggs and adult *Aedes aegypti* as an effort to determine natural larvicidal, ovicidal and repellant properties. *Citrus aurantifolia* is a *Citrus* genus consisting of chemical coumarins such as phenolics, scopoletin, flavonoids and limonin, which are favorable as phytochemical insecticide [38,39].

In the present study found that 0.01g/100ml water dilution was very effective to control *Aedes aegypti* 3$^{rd}$ and 4$^{th}$ instar larvae, 100% Knockdown within 60 minutes and 98.8% mortality within 24 hours followed by 0.005g dilution which was found 95.6% knockdown and 96.4% mortality and persistency was observed 100% ovicidal and larvicidal properties for 4 days. Muniandy and party revealed that same species of *Citrus aurantifolia* leaves decoction against larvae of *Aedes aegypti* the highest mortality was shown in 30% concentration with a total of 224 (74.6%) and in 90% concentration was 214 (71.3%) mortality. Probit analysis showed $LC_{50}$ was 38.5% and 6.6% at 24 and 48 hours, respectively. The highest rate of killing the larvae was taken at $LC_{60}$ with 91.6% for 24 hours and $LC_{65}$ 64.4% for the 48 hours by one way ANOVA [40]. Although in the present study mention that 0.01g peel essential oil dilution was a very effective to destroy nearly cent percent of mortality (98.8%) within 24 hours persistency was found 4 days. It was recommended to a study done in India, they revealed that the higher the concentration, the higher the mortality rate achieved by *Citrus sinensis*, a species of Genus *Citrus*. [41]. Research in Thailand8 has shown that *Citrus hystrix's* fruit peel has higher mortality compared to the plant against *Aedes aegypti* larvae (9) another study in Myanmar done on *Citrus hystrix's* fruit peel, Internal fruit materials and fruit extract were very effective to control *Aedes* larvae and repellent to control *Aedes* mosquito bite [42]. Another *Citrus* fruit peels essential oil dose 0.01g of orange peels essential oil produced 100% knockdown within 60minute and 100% mortality within 24hrs respectively and 100% ovicidal effect for 4days as well as persistency was observed 100% mortality of larvae for 4 days. The effective lethal concentrations $LC_{50}$ and $LC_{90}$ values were found to be 0.0015g and 0.0051g of peels essential oil (x=0.0418, P=0.05) was agreed with present study results of $LC_{50}$ and $LC_{90}$ values were found 0.0017 and 0.0053.Same result has been found in Indonesia study of *Citrus aurantifolia*

leaves decoction, $LC_{50}$ have shown 38.5% which is 385,470 ppm at 24-hour observation and 6.6% which is 66,130 ppm.

Furthermore, $LC_{90}$ could not be defined due to the highest killing effect of *Citrus aurantifolia* decoction, therefore, $LC_{60}$ has been taken with 91.6% which is 916,170 ppm for 24 hours and LC65 64.4% which is 644,940 ppm at 48 hours. Study in Nigeria12 has proved that Citrus species, Citrus sinensis has lower ppm with $LC_{50}$ 0.4 ppm and $LC_{90}$ with 0.9 ppm using ethanolic extraction method against *Aedes aegypti* larvae [40]. Another study showed that *Citrus limonia* and *Citrus Sinensis* fruit peels essential oils have high mortality against *Aedes aegypti* larvae with lower ppm, suggested that alcohol extract of used herbs contained more chemical components compared with decoction method [45-,46]. Singhi et al., [47] have reported that the latex of *Citrus procera* has shown larvicidal efficacy against all three important vector species as *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefasciatus* in India. The insecticidal activity of *Zingiber officinale* against *Anopheles pharoensis* 3rd stage showed 100% larval mortality rate [48]. Other study of bio-larvicide activity testing of orange peel, Lemon peel and Orange and Lemon peel essential oil mixture were analyzed and found that $LC_{50}$ and $LC_{90}$ values of lemon essential oil 0.001g and 0.004g found highest bio-larvicide property against 3rd and 4th instar *Aedes* larvae was more toxic and effective as bio-larvicide [49].

Sharma and his associate revealed that both leaves and peels essential oils of *Citrus aurantifolia* possess more ovicidal activity ($LC_{50}$ value of 5.26 ppm and 17.71 ppm for leaf and peel oil respectively at 72 hours) than larvicidal activity. Larvicidal activity showed rapid effect with $LC_{50}$ value of 128.81 ppm at 24 hours which reduced to 106.77 ppm at 72 hours while the leaf oil showed slow effect with $LC_{50}$ value of 188.59 ppm, 107.37 ppm and 104.59 ppm at 24 hours, 48 hours and 72 hours respectively. Again, the two essential oils did not show significant adulticidal activity. GC–MS analysis of both the oils recorded presence of different compounds. As a major constituent compound of the leaf Essential Oil of *Citrus aurantifolia*, Citral was tested for their ovicidal, larvicidal and adulticidal activities against *Aedes aegypti*. The result showed highest ovicidal activities ($LC_{50}$ value of 4.84 ppm at 72 hours) of Citral followed by larvicidal ($LC_{50}$ value of 87.02 ppm at 24 hours) and adulticidal ($LC_{50}$ value of 103.88 ppm at 24 hours) activities. The authors recommended that the essential oil extracted from the leaf and peel of *Citrus aurantifolia* and one of its major constituent compounds Citral can be included in the mosquito control programme of *Aedes aegypti* [50].

For repellency test 60 adult *Ae. aegypti* each was used against 0.04g of essential oil in 1ml EtOH diluent or 0.000128g/cm$^2$ each applied on 4 volunteer hands and found that 100% protection of *Aedes* mosquito landing to probe the skin. Repellency activity of complete protection time was observed 100% prevention for 120mins, over 90% prevention for 150 minutes and over 80% protection for 210minutes. Semi field trial observed that in the day time, it can prevent 3 hours of *Aedes* mosquito bite on oil applied areas of insect collectors in household. Same result has been found by same researcher using *Ocimum basilicum* L Leaves Essential Oil on *Aedes* mosquitoes in laboratory and simi field trial [51].

Abbas et al., [52] tested larvicidal, oviposition and repellent deterrent effects of essential oils (EOs) of *Mentha spicata* (L.), *Ocimum basilicum* (L.), and *Abutilon indicum* (L.) against three mosquito species (Diptera: Culicidae) including *Aedes aegypti* (L.), *Anopheles gambiae* s. l. Giles, and *Culex quinquefasciatus* Say by using

contact-based technique. In screening bioassays, *M. spicata* I, *M. spicata* II, *O. basilicum* I, *O. basilicum* II, and *A. indicum* EOs showed higher repellency against *Cx. quinquefasciatus* as compared to *Ae. aegypti* and *An. gambiae* when tested at 33.3 mg/cm$^2$. In time-span bioassays performed at 33.3 mg/cm2, EO of *M. spicata* I exhibited 100% repellence up to 45, 30, and 75 min against *Ae. aegypti*, *An. gambiae*, and *Cx. quinquefasciatus*, respectively.

Interestingly, at this tested dose, *M. spicata* I and *M. spicata* II showed higher repellence compared to DEET against *Ae. aegypti* and *Cx. quinquefasciatus* after 45 and 75 min, respectively. Their repellency was observed up to 150 and 210 min against *Ae. aegypti* and *Cx. quinquefasciatus*, respectively. In larvicidal bioassays, *M. spicata* I EO proved more toxic against 2$^{nd}$ instar larvae of *Ae. aegypti*, *An. gambiae*, and *Cx. quinquefasciatus* ($LC_{50}$ = 11.0, 42.9, and 12.6 mg/L, respectively) compared to other tested EOs were agreed with present study.

In the present study ovicidal properties of *Citrus aurantifolia* peel essential oil 0.01g in 100ml water could be ovicides 100% of *Aedes* eggs for 3 days. Although a researcher revealed that ovicidal activity of castor seed oil against *An. stephensi*, *Cx. fatigans* and *Ae. aegypti* mosquitoes successfully destroyed in laboratory [53]. The ovicidal activity of neem products *Azadirachtin* against mosquitoes *Cx. tarsalis* and *Cx. quinquefasciatus* was found potential effect of ovicidal on both mosquito eggs [54]. These ovicidal effects of the different plant's extracts were agreed with *Ocimum basilicum* leaves essential oil against *Aedes* eggs and also agreed with the ovicidal properties of *Citrus aurantifolia peel essential* on *Ae. aegypti* eggs. It may be due to the fact that the chemical constituents of *Citrus aurantifolia peels* essential oil like phenolics, scopoletin, flavonoids and limonin more effective phytochemical to destroyed eggs and larvae of *Aedes* mosquitoes [27,28]. In oviposition bioassays, *M. spicata* I exhibited the highest activity, showing 60%, 46%, and 79% oviposition deterrence against *Ae. aegypti*, *An. gambiae*, and *Cx. quinquefasciatus*, respectively, tested at a dose of 600 μg/cm$^2$. Major compounds of *M. spicata I*, *M. spicata* II, *O. basilicum* I, and *O. basilicum* II Eos were piperitenone oxide (38.8%), piperitone oxide (35.4%), estragole (55.3%), and linalool (43.8%), respectively and the author suggested that M. spicata EO could be used to control mosquitoes and their bites [52]. This finding of larvicidal, Ovicidal and repellency properties were agreed with the present finding of *Citrus aurantifolia* peel essential Oil against *Ae. aegypti*.

## 7.0. Conclusion

The study was conducted in laboratory for larvicidal, ovicidal and repellency properties of the indigenous plants fruit *Citrus aurantifolia* peels essential oil against *Aedes* adult, larvae and eggs. 0.01g of *Citrus aurantifolia* peels essential oil against 3$^{rd}$ and 4$^{th}$ instar larvae found 100% knockdown within 60minutes and 98.8% mortality within 24 hours and 98-100% of ovicidal and larvicidal persistency properties for 4 days. $LC_{50}$ and $LC_{90}$ values of *Citrus aurantifolia* peels essential oil was found to be 0.0017g and 0.0053g respectively. No toxicity effect and allergenicity were found in animal model and no irritation was found when testing repellency test. 0.04g /ml or 0.000128g/cm2 of *Citrus. aurantifolia* essential oil was found very effective to repel to protect 80 to 100% protection of *Ae. aegypti* mosquito bite for 210 minutes. Semi field trial observed that in the daytime, 100% prevention for 3 hours of *Aedes* mosquito bite on oil applied areas of insect collectors in household. Larvicidal, Ovicidal and repellency properties of the indigenous plants fruit *Citrus aurantifolia* peel essential oil *against Aedes aegypti* mosquito was found very effective to control *Aedes* Adult, larvae and eggs. Therefore, the essential oil can be reduced the dengue infection via to reduce the *Aedes* mosquito population and it could be suitable for the use in

mosquito control programme for a Public Health purpose. Study suggest that other plant base insecticides should be discovered in plants. And should be determined the effective insecticide ingredients in plant base extracts.

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**Competing Interests Statement**

The authors have declared that no competing financial, professional, or personal interests exist.

**Consent for publication**

All the authors contributed to the manuscript and consented to the publication of this research work.

**Authors' contributions**

All the authors took part in literature review, analysis, and manuscript writing equally.

**Availability of data and materials**

Supplementary information is available from the authors upon reasonable request.

**Ethical Approval**

Not applicable for this study.

**Institutional Review Board Statement**

Not applicable for this study.

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