



**LARVICIDAL ACTIVITIES OF PETROLEUM ETHER EXTRACTS OF DIFFERENT  
FRUIT PEEL WASTES AGAINST AN INDIAN STRAIN OF FILARIAL VECTOR, *CULEX  
QUINQUEFASCIATUS* SAY (DIPTERA: CULICIDAE)**

Shrankhla and Sarita Kumar\*

Department of Zoology, Acharya Narendra Dev College (University of Delhi) India.

\*Corresponding Author: Dr. Sarita Kumar

Department of Zoology, Acharya Narendra Dev College (University of Delhi) India.

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**ABSTRACT**

**Objective:** The larvicidal activity of the petroleum ether extracts of different fruit peels was assessed against the early fourth instar of *Culex quinquefasciatus* (*Cx. quinquefasciatus*). **Methods:** Petroleum ether extracts of peels of six common fruits were prepared and evaluated against early fourth instar larvae of *Cx. quinquefasciatus* using WHO standard procedure. The results were statistically analyzed to explore the most efficient extract. **Results:** Out of the six extracts, the *Citrus sinensis* and *Mangifera indica* peel extracts were found the most effective. Other tested fruit peel extracts resulted in 10-80% larval mortality after 24 to 48 hours of exposure. The larvicidal bioassays with *C. sinensis* resulted in LC<sub>50</sub> and LC<sub>90</sub> values were 31.345 ppm and 75.313 ppm, respectively as compared to high LC<sub>50</sub> and LC<sub>90</sub> values of 195.97 ppm and 633.05 ppm obtained with *M. indica*. **Conclusions:** The petroleum ether extract of *C. sinensis* peels was found as the most effective extract against early fourth instar of *Cx. quinquefasciatus* amongst the other tested fruit peel extracts. Further investigations are needed to identify the bioactive component and formulate control strategies.

**KEYWORDS:** *Citrus sinensis*, *Culex quinquefasciatus*, Fruit peels, Larvicidal potential, *Mangifera indica*.

**1.0 INTRODUCTION**

The year 2016 was a year of intense analysis over mosquito-borne diseases due to outbreak of various viral strains such as Zika, dengue, Chikungunya etc. In United States, in 2016, the West Nile Virus (WNV) was the most common virus transmitted to humans by mosquitoes. Over 150 species of mosquitoes have been known to transmit WNV by the vector species including *Culex pipiens*, *Cx. tarsalis*, *Cx. fatigans* and *Cx. quinquefasciatus*. It has also been reported that 47 out of 50 states and the District of Columbia were infected with West Nile virus in 2016. Globally, in 2016, a total of 2,038 cases of WNV were reported in humans with 94 confirmed deaths (4.61%)<sup>[1]</sup>. In addition, *Cx. tritaeniorhynchus*, *Cx. vishnui* and *Cx. pseudovishnui* group are the major vectors of Japanese Encephalitis (JE) leading to untimely and painful deaths, especially in children. Ministry of Health and Family Welfare, India has reported a total of 2034 cases of JE in 2017 (Till 24 December) leading to 230 fatalities, with maximum cases of 675 reported from the state of Uttar Pradesh<sup>[2]</sup>.

Apart from these, *Cx. quinquefasciatus* is a vector of a number of arboviruses and are a nuisance species in urban environments. In India, *Cx. quinquefasciatus* Say is the main vector of bancroftian filariasis, considered as

one of the major neglected diseases, caused by *Culex*-transmitted filarial nematodes, *Wuchereria bancrofti* and *Brugia malayi*. Approximately, 1.2 billion individuals; which is about 20% of the world's population; are at risk of acquiring filarial infection. It is important to note that one third of these individuals live in India, one third in Africa and most of the remainder in Asia, the Pacific and Americas<sup>[3]</sup>. As per Government of India reports, a large number of indigenous cases of filariasis have been reported from about 256 districts in 213 states/Union territories<sup>[4]</sup>.

*Culex quinquefasciatus*, like many other arthropod species, is challenging to control due to its emerging resistance to synthetic insecticides; such as organochlorines, organophosphates, carbamates, pyrethroids etc.<sup>[5, 6]</sup> Furthermore, insecticides-based control measures are causing an adverse impact on the environment, non-target organisms and human health. Therefore, we made an attempt to develop biodegradable and effective mosquito larvicides formulated from plant waste products. It would not only help in to formulate mosquito control strategies but also help us to recycle these wastes. Consequently, plants have attracted the consideration of many researchers, and plant extracts or essential oils prepared from the fruit peels are under

investigations for various purposes, especially against control of mosquitoes. Many plants have been observed to contain bioactive components, which may prove to help the control of insects and for field applications in mosquito control programs<sup>[7]</sup>. Limonene isolated from the abraded fresh peels of *Citrus aurantium* has been studied for its larvicidal toxicity against *Cx. quinquefasciatus*<sup>[8]</sup>. The larvicidal efficacy of *Citrus bergamia* essential oil has been confirmed against *Ae. aegypti* and *Cx. pipiens*<sup>[9]</sup>. The essential oils extracted from *C. limon* has been investigated against three mosquito species: *Aedes aegypti*, *Cx. quinquefasciatus* and *Anopheles stephensi* and found effective<sup>[10]</sup>. Likewise, Kumar *et al.* investigated peel extracts of *C. limetta* and assayed against *Ae. aegypti* and *An. stephensi* and reported significant efficacy<sup>[11]</sup>.

Keeping this in view, we investigated peels of six common fruits, extracted in petroleum ether and assayed against early fourth instars of *Cx. quinquefasciatus*. The utilization of fruit peel wastes in the form of anti-mosquito products would not only assist to decrease waste load but would also reduce the pollution load and recover the environmental profile of fruit juice processing industry.

## 2.0 MATERIALS AND METHODS

### 2.1 Collection of Plant Material

The fruits of *Citrus sinensis* (Orange), *Citrus limetta* (Sweet lime), *Citrus limon* (Lemon), *Cucumis melo* (Muskmelon), *Mangifera indica* (Mango) and *Musa paradisiaca* (Banana) were collected from the surrounding markets and nearby juice shops. The peels were separated from the fruits and thoroughly washed with tap water to remove the dust and other particles from the peels. Healthy and fresh peels were separated and kept under shade at the room temperature (28±1) °C for about 15 to 20 days till they dried completely. Utmost care was taken to prevent any fungal growth or they were discarded.

### 2.2 Preparation of Peel Extracts

The dried peels were crushed manually or grinded mechanically using a blender mixture. The 20 g of each dried peel was extracted in 200 mL of petroleum ether solvent separately in a Soxhlet apparatus (Borosil, India) for up to 72 h (8 h per day) at the temperature not exceeding the boiling point of the solvent. Crude extracts were concentrated using a vacuum rotary evaporator at 45°C under low pressure and a semisolid residue was obtained. The residue was stored in refrigerator at 4°C until tested. The 1,000 ppm of stock solution was prepared for investigations.

### 2.3 Rearing of Culicine mosquitoes

The filarial vector, *Cx. quinquefasciatus* was collected from the surroundings of Delhi region. The colony was maintained in an insectary at 28±1 °C temperature and 80±5% relative humidity<sup>[12]</sup>. Freshly soaked raisins were supplied to the adult mosquitoes. Blood meals were

periodically provided to female mosquito for the maturation of eggs. The egg rafts were allowed to lay in water kept in a bowl in the cages and then transferred into the enamel trays for hatching. Larvae usually hatched within 36 h and were fed with larval food (Yeasts and Dog biscuits in 1:3 ratio). Formation of pupae started after 12-13 days which were then transferred into the cloth cages for adult emergence.

### 2.4 Screening of Peel Extracts for Larvicidal Potential against *Culex quinquefasciatus*

The larvicidal bioassay was performed at 28±1 °C on the *Cx. quinquefasciatus* early fourth instars in accordance with the procedure by WHO with some modifications<sup>[13]</sup>. The peel extracts were diluted by using ethanol as a solvent. Bioassays were conducted separately in 500 mL glass beakers containing 1 mL of the test concentration (1000 ppm) and 249 mL water. Twenty larvae (early fourth instar) of *Cx. quinquefasciatus* were evaluated for each test concentration of the extracts according to WHO standard procedures. Three replicates were conducted for each test. Simultaneously, controls were run parallel in triplicate with 1 mL of ethanol mixed with 249 mL of water at 28±1°C and 80–90% relative humidity (RH). The moribund and dead larvae in all sets were counted after 24 h and 48 h, and expressed as per cent larval mortality for each concentration. Dead larvae were defined as those that failed to move after probing with a needle, while moribund larvae were defined as those incapable of rising to the surface within a reasonable period of time or not demonstrating characteristic diving reactions when the water was disturbed. Replicates with >20% mortality in the controls were discarded and repeated. Mortality values ranging 5–20% in the controls were corrected using Abbott's formula<sup>[14]</sup> as follows: Corrected % mortality =  $([T - C] / [100 - C]) \times 100$  where *T* is the per cent mortality in the test concentrations and *C* is the per cent mortality in the control.

### 2.5 Larvicidal Bioassay

The extracts that failed to give 100% mortality after 24 h exposure, were not investigated further. The extracts that inflicted 100% mortality after contact for less than 24 h were selected and used for the next stage of the study. The larvae were assayed with selected peel extracts to calculate the lethal concentrations which result in at least 50% larval mortality. The larvicidal bioassay was performed as described in the previous section. The graded series of each of the selected extracts was prepared using ethanol as the solvent. Three replicates were carried out simultaneously for each dilution making a total of 60 larvae for each concentration of each extract. Controls were exposed to the solvent, i.e. ethanol alone.

### 2.6 Data analysis

All the data observed were subjected to Probit analysis for calculating concentrations that produced 50% mortality (LC<sub>50</sub>) and LC<sub>90</sub> values and other statistical

parameters at 95% confidence levels using SPSS software.

### 3.0 RESULTS AND DISCUSSION

The present investigation was carried out with an objective to develop an ecofriendly and cost effective phytopesticide which would provide an alternate to harmful synthetic insecticides and boost environment profile<sup>[16]</sup>. Many researches on vector control have exposed the efficiency of different phytochemicals obtained from various plants against different species of mosquitoes<sup>[17, 18]</sup>. Keeping this objective in view, the current investigations were carried out to explore certain fruit peels for the possible agents of *Cx. quinquefasciatus* control. The utilization of fruit peels would not only help in the minimization of fruit wastes from our country to a large extent but also will be productive to control the mosquito population. Subsequently, the petroleum ether

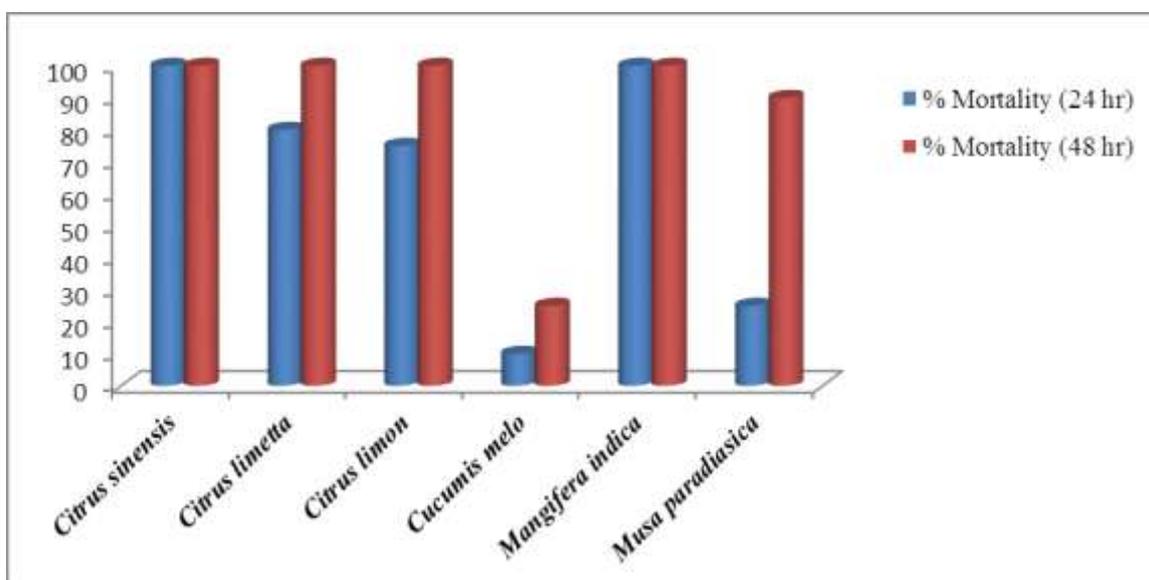
extracts of peels of six common fruits; *C. sinensis*, *C. limetta*, *C. limon*, *C. melo*, *M. indica*, *M. paradisiaca*; were tested for their larvicidal efficacy against the early fourth instar larvae of *Cx. quinquefasciatus*.

The relative efficacy of the petroleum ether extract of different fruit peels wastes is presented in Table 1. The 1000 ppm of extracts were tested against early fourth instar larvae of *Cx. quinquefasciatus* for 24 and 48 h of exposure. The peel extracts of *C. sinensis* and *M. indica* showed 100% larvicidal efficacy within 24 h of exposure. During the bioassay, no pupal formation and adult emergence was observed indicating the accuracy of larval selection. Other extracts were not effective to a great extent and resulted in 10-80% larval mortality, the least effective extract was observed to be *C. melo* petroleum ether extract. Nevertheless, the efficacy of *C. melo* increased to 25% after another day of exposure.

**Table 1: Biological evaluation of petroleum ether extracts of peel wastes of six fruits against *Culex quinquefasciatus*.**

Name of plant species	Local Names	Family	% Mortality in 24 h	% Mortality in 48 h
<i>Citrus sinensis</i>	Orange	Rutaceae	100%	100%
<i>Citrus limetta</i>	Mausambi	Rutaceae	80%	100%
<i>Citrus limon</i>	Lemon	Rutaceae	75%	100%
<i>Cucumis melo</i>	Musk Melon	Cucurbitaceae	10%	25%
<i>Mangifera indica</i>	Mango	Anacardiaceae	100%	100%
<i>Musa paradisiaca</i>	Banana	Musaceae	25%	90%

On the other hand, *C. limetta* peel extracts inflicted 80% mortality in 24 h which increased to 100% when the assay was extended for another day (Table 1; Fig. 1). Likewise, *C. limon* exhibited 75% and 100% mortality after 24 h and 48 h of exposure, respectively. In contrast, *Musa paradisiaca* peels extracted in petroleum ether resulted in 25% and 90% mortality after respective exposure of 24 h and 48 h (Fig. 1).



**Fig. 1: Relative bioefficacy of petroleum ether extracts of fruit peel wastes against filarial vector, *Culex quinquefasciatus*.**

The screening results clearly show that petroleum ether extracts of the *C. sinensis* and *M. indica* peels were the most efficient extracts against early fourth instars of *Cx. quinquefasciatus*. Therefore, these extracts were investigated further for their larvicidal potential. The larvicidal bioassay results with  $LC_{50}$  and  $LC_{90}$  values are presented in Table 2. When

early fourth instars of *Cx. quinquefasciatus* were assayed with petroleum ether extract of *C. sinensis* for 24 h, the LC<sub>50</sub> value obtained was 31.35 ppm while the LC<sub>90</sub> value was 75.31 ppm (Table 2). In contrast, the bioassay with *M. indica* resulted in quite high LC<sub>50</sub> and LC<sub>90</sub> values of 195.97 ppm and 633.05 ppm, respectively (Table 2).

**Table 2: Larvicidal potential of petroleum ether extract of *Citrus sinensis* and *Mangifera indica* peels against *Culex quinquefasciatus*.**

Plant species	LC <sub>50</sub> (ppm)	95% Fiducial limits	LC <sub>90</sub> (ppm)	95% Fiducial limits	S.E.	χ <sup>2</sup> (df)	R.C.
<i>Citrus sinensis</i>	31.35	21.99 - 40.92	75.31	54.87 - 148.91	0.78	2.28	3.37
<i>Mangifera indica</i>	195.97	134.79 - 262.38	633.05	430.42 - 1443.45	0.55	3.55	2.52

No mortality was observed in the control

LC<sub>50</sub> - lethal concentration that kills 50 % of the exposed larvae, LC<sub>90</sub> - lethal concentration that kills 90 % of the exposed larvae

S.E. = Standard error, χ<sup>2</sup> = chi-square, df = degree of freedom, R.C. = Regression Coefficient

Test samples were transformed into log covariant (log<sub>10</sub>), p>0.05, level of significance is greater than 0.05, no heterogeneity factor is used in the calculation of confidence limits, Values are mean of three replicates

Many researches were carried out on the fruit peel wastes by different investigators. Our results are in agreement with that of Murugan *et al.* who found *C. sinensis* ethanolic peel extracts effective against *Cx. quinquefasciatus*, though the LC<sub>50</sub> value obtained by them was quite high (244.70 ppm) as compared to obtained in our investigations<sup>[19]</sup>. The hexane extracts of *C. limetta* peels was studied against *An. stephensi* and *Ae. aegypti* which resulted in LC<sub>50</sub> values of 132.45 and 96.15 ppm, respectively<sup>[11]</sup>. These investigations revealed that hexane extracts of *C. limetta* peels exhibited 1.9 and 1.5-fold more larvicidal potential than the petroleum ether extracts against early fourth instars of *An. stephensi* and *Ae. aegypti*. Chloroform extracts of orange peel demonstrated 58.25 ppm as LC<sub>50</sub> value and 298.31 ppm as LC<sub>90</sub> value against *An. subpictus*<sup>[20]</sup>. Prior studies have evidently proved the excellent larvicidal efficacy of *C. aurantium*, *C. sinensis* and *C. lemon* peel oils against *Cx. quinquefasciatus*, the lemon peel oil was assessed as the best larvicidal agent<sup>[21, 22]</sup>.

The volatile peel extract of *C. sinensis* and *C. aurantifolia* were also tested and reported to have insecticidal activity against mosquito larvae<sup>[23]</sup>. Akram *et al.* evidently proved the efficacy of seed extracts of rough lemon and lemon against *Ae. albopictus* with respective LC<sub>50</sub> values of 119.993 ppm and 137.258 ppm, respectively, after 24 h of exposure and 108.85 and 119.853 ppm, respectively, after 48 h of exposure<sup>[24]</sup>. Earlier, Amusan *et al.* revealed the potency of the ethanolic extracts of *C. sinensis* against the larvae of the yellow fever mosquito<sup>[25]</sup>.

#### 4.0 CONCLUSION AND RECOMMENDATIONS

Our studies have clearly demonstrated that waste peels of fruits can be utilized as the effective agents of mosquito control. Peel waste though degradable and seasonal poses a massive problem of disposable to the fruit juice processing industries and pollution monitoring agencies. Reutilizing of fruit waste to control the mosquito population would help in minimizing these wastes and

would boost environment profile by management of wastes. However, further investigations are required to identify bioactive constituents in these extracts to formulate mosquito control strategies. Investigations of more fruit wastes and extraction in different solvents may provide more insights into this area.

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